METAGENOMIC DOMAIN SUBSTITUTION FOR THE HIGH-THROUGHPUT CREATION OF NON-RIBOSOMAL PEPTIDE ANALOGUES

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Non-ribosomal peptides are a diverse and medically important group of natural products. They are biosynthesised by modular non-ribosomal peptide synthetase (NRPS) assembly lines in which domains from each module act in concert to incorporate a specific amino acid into a peptide. Each module is comprised of domains which catalyse substrate recognition and activation (adenylation, or A-domain), peptide bond formation (condensation, or C-domain); and substrate loading and transfer (thiolation, or T-domain). This modular biosynthesis has driven enzyme engineering efforts to generate new peptide analogues by substituting amino acid-specifying domains. Rational NRPS engineering has increasingly focused on using recombination sites favoured by evolution for domain substitution. Rational approaches have the drawback of requiring constructs to be designed and tested individually with variable yields and success rates. Here, we present an alternative evolution-inspired approach, focused on large-scale diversification and screening, to overcome a low efficiency of generating functional recombinant enzymes by sheer volume of recombination events. By amplifying NRPS domains from soil-derived metagenomic DNA at conserved motif sequences, we leveraged natural diversity for parallel substitution of over 1,000 unique sequences into a model pyoverdine synthetase. Screening via fluorescence and mass spectrometry followed by sequencing, identified over 100 functional domain substitutions that collectively yielded 16 unique pyoverdines as major products. This metagenomic approach shifts the focus of engineering non-ribosomal peptide biosynthesis from requiring a high success rate of individual domain substitutions to developing effective high-throughput screens, offering a novel approach to generating new drug candidates that are inaccessible to conventional chemistry.

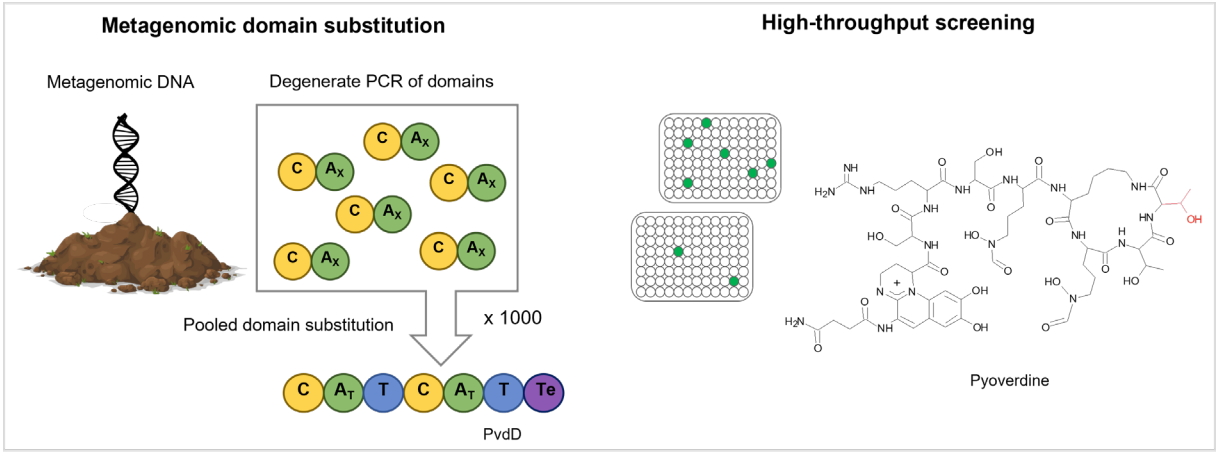

Figure 1. Metagenomic domain substitution involves amplification of uncharacterized pools of NRPS domains from metagenomic DNA using degenerate primers which are then used for substitution. High-throughput screening is carried out to identify bacterial strains producing modified non-ribosomal peptides. This study targeted the 2nd module of PvdD for substitutions, which incorporates the terminal L-threonine residue, highlighted in red, into pyoverdine. A_T refers to threonine-specific A domains; A_X refers to A domains that have specificity for unknown amino acids.